

CLAIMS

Sub C3

1. A method for identifying a subunit specific modulator of the N-methyl-D-aspartate (NMDA) receptor, comprising:
  - a) providing a plurality of NMDA receptors which differ in their subunit identity;
  - b) contacting the NMDA receptors of step a) with a neurotransmitter recognition site ligand in the presence and absence of a candidate modulator; and
  - c) assaying for receptor activity following step b), wherein an increase or decrease in activity in at least one, but not all members of the plurality of NMDA receptors, in the presence but not the absence of a candidate modulator, is an indication that the candidate modulator is a subunit specific modulator.
2. The method of Claim 1 further comprising comparing the subunit identity of the subset of the NMDA receptors to determine the subunit specificity of the candidate modulator.
3. The method of Claim 1 wherein the plurality of NMDA receptors have identical NR2 subunits, and differ in their NR1 subunits.
4. The method of Claim 3 wherein the identical NR2 subunits are selected from the group consisting of NR2A, NR2B, NR2C, and NR2D.
5. The method of Claim 3 wherein at least one of the NR1 subunits is a natural isoform selected from the group consisting of NR1<sub>000</sub>, NR1<sub>001</sub>, NR1<sub>010</sub>, NR1<sub>011</sub>, NR1<sub>100</sub>, NR1<sub>101</sub>, NR1<sub>110</sub>, and NR1<sub>111</sub>.

*Part E2*

Det E 3

6. The method of Claim 3 wherein at least one of the NR1 subunits contain an  $\alpha$  exon encoded protein domain.
7. The method of Claim 3 wherein at least one of the NR1 subunits is a chimeric isoform.
8. The method of Claim 3 wherein at least one of the NR1 subunits is an isoform point mutant.
9. The method of Claim 8 wherein the point mutant contains at least one point mutation at a residue which corresponds to residue 182, 193, 202, 233, or 252 of NR1<sub>011</sub>.
10. The method of Claim 9 wherein the isoform point mutant is a penta-mutant with the amino acid substitution mutations which correspond to mutations R182A, K193A, K202A, R233A, and R252A of NR1<sub>011</sub>.
11. The method of Claim 8 wherein the isoform point mutant contains an  $\alpha$  exon encoded protein domain and has point mutations within that domain.
12. The method of Claim 1 wherein the plurality of NMDA receptors have identical NR1 subunits, and differ in their NR2 subunits.
13. The method of Claim 12 wherein the identical NR1 subunits are an isoform selected from the group consisting of NR1<sub>000</sub>, NR1<sub>001</sub>, NR1<sub>010</sub>, NR1<sub>011</sub>, NR1<sub>100</sub>, NR1<sub>101</sub>, NR1<sub>110</sub>, and NR1<sub>111</sub>.
14. The method of Claim 12 wherein the identical NR1 subunits contain an  $\alpha$ -exon encoded protein domain.

15. The method of Claim 12 wherein the identical NR1 subunits are a chimeric isoform.
16. The method of Claim 12 wherein the identical NR1 subunits are an isoform point mutant which contains an  $\alpha$  exon encoded protein domain and has point mutations within that domain.
17. The method of Claim 16 wherein the identical NR1 subunits contain an  $\alpha$  exon encoded protein domain.
18. The method of Claim 17 wherein the identical NR1 subunits are point mutants which contain at least one point mutation at a residue which corresponds to residue 182, 193, 202, 233, or 252 of NR1<sub>011</sub>.
19. The method of Claim 18 wherein the identical NR1 subunits are a penta-mutant with the amino acid substitution mutations which correspond to mutations R182A, K193A, K202A, R233A, and R252A of NR1<sub>011</sub>.
20. The method of Claim 12 wherein at least one of the NR2 subunits is an isoform selected from the group consisting of NR2A, NR2B, NR2C, and NR2D.
21. The method of Claim 12 wherein at least one of the NR2 subunits is a chimeric isoform.
22. The method of Claim 21 wherein the chimeric isoform contains a.a. 534-870 of NR2B.
23. The method of Claim 22 wherein the chimeric isoform contains amino acid 548-892 of NR2D.

24. The method of Claim 22 wherein the chimeric isoform contains amino acid 703-870 of NR2B.

25. The method of Claim 12 wherein at least one of the NR2 subunits is an isoform point mutant.

~~26.~~<sup>6</sup> The method of Claim 1 wherein assaying step c) is with an oocyte expression system.

~~27.~~<sup>6</sup> The method of Claim 1 wherein the neurotransmitter recognition site ligand is an agonist.

~~28.~~<sup>6</sup> The method of Claim ~~27~~<sup>6</sup> wherein the agonist is selected from the group consisting of NMDA, glutamate, and glycine.

~~29.~~<sup>8</sup> The method of Claim 1 wherein the neurotransmitter recognition site ligand is an antagonist.

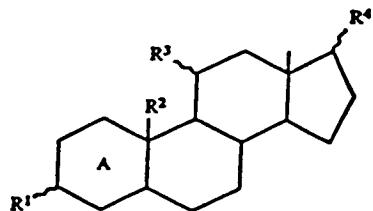
30. The method of Claim 1 wherein the candidate modulator is a steroid based molecule.

31. The method of Claim 1 wherein the candidate modulator is a non-steroid based molecule.

~~32.~~<sup>9</sup> The method of Claim 1 wherein the candidate modulator is obtained from a library of small molecules.

~~33.~~<sup>10</sup> The method of Claim 1 wherein the candidate modulator is a known neuromodulator.

34. A method for inhibiting N-methyl-D-aspartate glutamate receptor mediated ion-channel activity in an individual in need thereof comprising administering an effective amount of a compound represented by the following structural formula:



wherein:

ring A has 0-3 double bonds;  
R<sup>1</sup> is -OH, =O, or a negatively charged group;  
R<sup>2</sup> is -H, -CH<sub>3</sub>, or is absent when ring A has three double bonds;  
R<sup>3</sup> is -H, OH, =O, or -OR';  
R<sup>4</sup> is an aliphatic or aromatic group; and  
R<sup>4</sup> is -OH, =O or -COCH<sub>3</sub>.

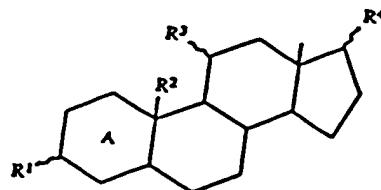
35. The method of Claim 34 wherein R<sup>1</sup> is either hemioxylate, hemisuccinate, or hemiglutarate.

36. The method of Claim 35 wherein the compound is selected from the group consisting of pregnanolone hemioxylate (3 $\alpha$ 5 $\beta$ HO), pregnanolone hemisuccinate (3 $\alpha$ 5 $\beta$ HS), and pregnanolone hemiglutarate (3 $\alpha$ 5 $\beta$ HG).

37. The method of Claim 36 wherein the effective amount is a concentration of from about 1 to about 500  $\mu$ M.

38. The method of Claim 37 wherein the effective amount is from about 50 to about 250  $\mu$ M.

39. A method for inhibiting the toxic effects associated with activation of the N-methyl-D-aspartate receptor in neurons in an individual in need thereof, comprising administering an effective amount of a compound represented by the following structural formula:



wherein:

ring A has 0-3 double bonds;  
 $R^1$  is  $-OH$ ,  $=O$ , or a negatively charged group;  
 $R^2$  is  $-H$ ,  $-CH_3$ , or is absent when ring A has three double bonds;  
 $R^3$  is  $-H$ ,  $OH$ ,  $=O$ , or  $-OR'$ ;  
 $R^1$  is an aliphatic or aromatic group; and  
 $R^4$  is  $-OH$ ,  $=O$  or  $-COCH_3$ .

40. The method of Claim 39 wherein  $R^1$  is either hemioxylate, hemisuccinate, or hemiglutarate.

41. The method of Claim 40 wherein the compound is selected from the group consisting of pregnanolone hemioxylate ( $3\alpha 5\beta HO$ ), pregnanolone hemisuccinate ( $3\alpha 5\beta HS$ ), and pregnanolone hemiglutarate ( $3\alpha 5\beta HG$ ).

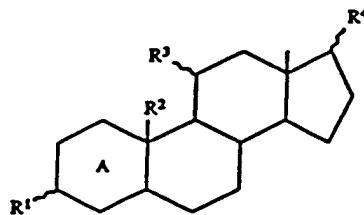
42. The method of Claim 39 wherein the effective amount is a concentration of from about 1 to about  $500 \mu M$ .

43. The method of Claim 42 wherein the effective amount is from about 50 to about  $250 \mu M$ .

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44. The method of Claim 39 wherein the neurons are selected from the group consisting of hippocampal cells and spinal cord cells.

45. A method for reducing neuronal cell death resulting from L-glutamate activation of the N-methyl-D-aspartate receptor in an individual in need thereof, comprising administering an effective amount of a compound represented by the following structural formula:



wherein:

ring A has 0-3 double bonds;  
R<sup>1</sup> is -OH, =O, or a negatively charged group;  
R<sup>2</sup> is -H, -CH<sub>3</sub>, or is absent when ring A has three double bonds;  
R<sup>3</sup> is -H, OH, =O, or -OR';  
R<sup>1</sup> is an aliphatic or aromatic group; and  
R<sup>4</sup> is -OH, =O or -COCH<sub>3</sub>.

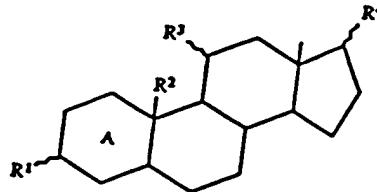
46. The method of Claim 45 wherein R<sup>1</sup> is either hemioxylate, hemisuccinate, or hemiglutarate.

47. The method of Claim 46 wherein the compound is selected from the group consisting of pregnanolone hemioxylate (3 $\alpha$ 5 $\beta$ HO), pregnanolone hemisuccinate (3 $\alpha$ 5 $\beta$ HS), and pregnanolone hemiglutarate (3 $\alpha$ 5 $\beta$ HG).

48. The method of Claim 45 wherein the effective amount is a concentration of from about 1 to about 500  $\mu$ M.

49. The method of Claim 48 wherein the effective amount is from about 50 to about 250  $\mu$ M.

50. A method for treating a disease selected from the group consisting of neuropathic pain, drug withdrawal/dependency, epilepsy, glaucoma, chronic neurodegenerative diseases, amyotrophic lateral sclerosis, anxiety disorders, brain cell death, ischaemia, stroke, and trauma in an individual when said disease results from agonist induced NMDA receptor activation comprising administering to the individual an effective amount of a compound represented by the following structural formula:



wherein:

ring A has 0-3 double bonds;  
 $R^1$  is  $-OH$ ,  $=O$ , or a negatively charged group;  
 $R^2$  is  $-H$ ,  $-CH_3$ , or is absent when ring A has three double bonds;  
 $R^3$  is  $-H$ ,  $OH$ ,  $=O$ , or  $-OR'$ ;  
 $R^1$  is an aliphatic or aromatic group; and  
 $R^4$  is  $-OH$ ,  $=O$  or  $-COCH_3$ .

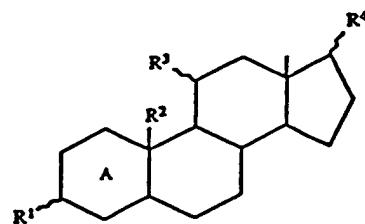
51. The method of Claim 50 wherein  $R^1$  is either hemioxylate, hemisuccinate, or hemiglutarate.

52. The method of Claim 51 wherein the compound is selected from the group consisting of pregnanolone hemioxylate ( $3\alpha 5\beta HO$ ), pregnanolone hemisuccinate ( $3\alpha 5\beta HS$ ), and pregnanolone hemiglutarate ( $3\alpha 5\beta HG$ ).

53. The method of Claim 50 wherein the effective amount is a concentration of from about 1 to about 500  $\mu$ M.

54. The method of Claim 53 wherein the effective amount is from about 50 to about 250  $\mu$ M.

55. A method for inhibiting the excitatory L-glutamate-mediated synaptic activity in an individual in need thereof, comprising administering to the individual a compound represented by the following structural formula:



wherein:

ring A has 0-3 double bonds;

$R^1$  is  $-OH$ ,  $=O$ , or a negatively charged group;

$R^2$  is  $-H$ ,  $-CH_3$ , or is absent when ring A has three double bonds;

$R^3$  is  $-H$ ,  $OH$ ,  $=O$ , or  $-OR'$ ;

$R^1$  is an aliphatic or aromatic group; and

$R^4$  is  $-OH$ ,  $=O$  or  $-COCH_3$ .

56. The method of Claim 55 wherein  $R^1$  is either hemioxylate, hemisuccinate, or hemiglutarate.

57. The method of Claim 56 wherein the compound is selected from the group consisting of pregnanolone hemioxylate ( $3\alpha 5\beta HO$ ), pregnanolone hemisuccinate ( $3\alpha 5\beta HS$ ), and pregnanolone hemiglutarate ( $3\alpha 5\beta HG$ ).

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